AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY 1:246-254 (1981)
0271-7352/81/0105-024683.00 © 1981 ALAN R. LISS, INC.

AJRI Pregnancy Immunology

Human Trophoblast-Specific Surface Antigens Identified Using Monoclonal Antibodies*

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ABSTRACT: **Mouse monoclonal antibodies have been produced against syncytiotrophoblast plasma membrane preparations isolated from term human placentas. Of 20 positive clones, two antibodies (H309, H318) were directed against the Cγ2 domain of IgG, one (H312) was directed against albumin, and another (H303) was directed against a further normal human serum component (not transferrin). One monoclonal antibody (H310) recognized an antigenic epitope restricted to trophoblast and lymphocytes: this antibody did not inhibit mitogenic or allogeneic stimulation of lymphocytes. Two monoclonal antibodies (H315 and H317) reacted with trophoblast-specific antigenic determinants. The H315 antigen was present on first trimester syncytiotrophoblast, unlike the H317 antigen. (Am J Reprod Immunol. 1981; 1:246-254.)**

Key words: Hybridomas, monoclonal antibodies, trophoblast antigens, placental syncytiotrophoblast, lymphocytes, immunofluorescence, ELISA.

INTRODUCTION

The syncytiotrophoblast microvillous plasma membrane (StMPM) of the human placenta is the effective maternofetal interface, and molecular components expressed on this membrane are known to function in invasive, immunological, and molecular recognition processes essential to the welfare and maintenance of pregnancy.[1,2] In agreement, biochemical studies have shown multiple molecular components associated with StMPM,[3,4] although major histocompatibility antigens are not significantly expressed at this site.[1,2] Some StMPM-associated components, such as placental alkaline phosphatase or the receptor for transferrin, have now been well documented.[3-6] However, many StMPM-associated components remain unidentified and their more detailed characterization might be expected to uncover further avenues of interest within pregnancy immunology.

The advent of somatic cell hybridization technology to achieve in vitro production of monoclonal antibodies[7-9] has introduced new scope for methods aimed at the finite analysis of complex surface components of multifunctional cells. Thus, we have used a "shotgun" approach in the production of mouse monoclonal antibodies against unsolubilized StMPM vesicle preparations isolated from human term placentas; the separate specificities of these monoclonal antibodies have then been analyzed by extensive immunofluorescence and immunoassay procedures. This study has confirmed the presence of human trophoblast-specific antigenic epitopes recognized by heterologous immunization, and has also offered evidence indicating the presence of an antigenic epitope common only to trophoblast and lymphocytes.

Submitted for publication May 8, 1981; accepted May 20, 1981.
*This work was supported in part by the North West Cancer Research Fund, and was presented in part at the Second International S. B. Gusberg Symposium on Reproductive Immunology, New York, June 1981.
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MATERIALS AND METHODS

Syncytiotrophoblast Microvillous Plasma Membrane Preparations

Syncytiotrophoblast microvillous plasma membrane vesicle preparations were isolated from fresh normal term human placental villous tissue by cold saline extraction as described in detail previously.[3,4,10] When required for enzyme-linked immunosorbent assay (ELISA), intact StMPM vesicles were solubilized with 1% sodium deoxycholate (NaDOC) in 10 mM Tris-HCl, pH 8.2.[3] For comparative ELISA experiments, crude plasma membrane preparations were also prepared following thorough washing and homogenization of normal human kidney tissue and subsequent differential ultracentrifugation to achieve a membrane pellet as performed for placental tissue extracts.

Immunization and Production of Hybridomas

BALB/c mice were immunized by two intravenous injections (days 0 and 14) of 500 μg unsolubilized pooled StMPM preparation isolated from two or three separate placentas. On day 17, spleen cells were fused with the P3-NSI Ag-1 mouse plasmacytoma cell line using polyethylene glycol 1500.[11] Cells were then seeded at 10^6 cells/well in hypoxanthine, aminopterin, and thymidine (HAT) selection medium dispensed in flat-bottomed microtiter plates. After seven days, the cultures were maintained in HT medium, and screened at 21 days for antibody against StMPM using ELISA. Cells were grown up from 20 positive wells and stored in liquid nitrogen. These were designated H300-H319, and all studies of monoclonal antibody specificities were performed on spent hybridoma culture supernatants. Hybridoma cultures H309, H310, H315, H316, H317, H318, and H319 have now been recloned three times by limiting dilution in microtiter plates.

Immunofluorescence Studies on Tissue Sections

Indirect immunofluorescence on cryostat sections of normal fresh term or first trimester human placental villous tissue was performed.[12,13] Appropriate dilutions of each hybridoma culture supernatant were used as sources of monoclonal antibody in the first layer and a fluorescein isothiocyanate (FITC)-conjugated rabbit antibody to mouse immunoglobulin (Ig; Miles Research Laboratories,

Slough, UK) adsorbed with Sepharose-immobilized normal human serum (NHS)[14,15] was used at 1:60 dilution in the second layer. Controls, fluorescence microscopy, and photomicrography were performed as described previously.[14,15] Umbilical cord, amniotic, and chorionic membranes were also collected at delivery; the latter membranes were snap-frozen around a small piece of rat liver for support. Rheumatoid synovial membranes were collected at synovectomy and 22 separate small pieces of adult human tissues were obtained at postmortem within six hours of death; these were processed immediately for immunofluorescence as for placental tissue.

Cell Surface Immunofluorescence

Human peripheral blood mononuclear leukocytes (PBL) were separated from heparinized venous blood of healthy donors by density centrifugation on Lymphoprep (Nyegaard A/S, Oslo). Cell surface immunofluorescence was performed on washed PBL by incubation of $1-2 \times 10^5$ cells at 4 °C for 30-60 min with 50 μl of appropriate dilutions of each decomplemented hybridoma culture supernatant in medium 199 with 0.02% azide. The cells were washed twice at 4 °C and then incubated at 4 °C for 30-60 min with a 1:20 dilution of the FITC-conjugated antimouse Ig antibody. After two washes, the cells were incubated for 10 min at 4 °C with 50 μl of a 1:10 dilution of 50 μg/ml propidium iodide (Sigma Chemical, St Louis). This was performed in order to assess viability simultaneously with fluorescence. Cells were finally washed twice and examined by fluorescence microscopy with epiillumination using oil-immersion objectives. Cell surface immunofluorescence studies were performed as for human PBL on cells from the MCF-7 human mammary carcinoma cell line maintained in culture in this laboratory.[4,15]

Lymphocytotoxicity Studies

Complement-dependent lymphocytotoxicity was determined by a microtechnique using Terasaki plates in which 1 μl of each decomplemented hybridoma culture supernatant was incubated for 60 min at room temperature with a panel of 1-μl suspensions of normal human PBL (2×10^6 cells/ml) in RPMI-1640. After one wash, 5 μl of rabbit complement was added and the plates incubated at 37 °C for 30 min. Viability was then assessed following addition of 1 μl 5% eosin and 1 μl neutral 37% formaldehyde.

Lymphocyte Responsiveness Assays

The effect of adding 50 μl of each decomplemented and dialyzed hybridoma culture supernatant (or culture medium alone) was determined on two-way mixed lymphocyte culture (MLC) reactions and on phytohemagglutinin (PHA)-induced stimulation of lymphocytes. Mixed lymphocyte culture reactions were performed by addition of 100 μl PBL (2×10^6 cells/ml) with 100 μl unrelated PBL (2×10^6 cells/ml) in Dulbecco's modified Eagle's medium with 10% heat-inactivated AB serum in round-bottomed microtiter plates. Cultures were prepared in quadruplicate and incubated in a humidified 5% CO_2 incubator at 37 °C for five days. Then ^{3}H-thymidine was added (1 μCi/well) and the cells harvested 18 h later with a multiple sample harvester. Incorporation of radioactivity was assessed in a RackBeta automatic counter (LKB, Sweden). Phytohemagglutinin stimulation was performed similarly by incubation of 200 μl PBL (2×10^6 cells/ml) with 20 μl 100μg/ml purified PHA (Wellcome Reagents, Beckenham, UK) for two days prior to addition of ^{3}H-thymidine.

Erythrocyte Agglutination Studies

Human erythrocyte (RBC) direct agglutination was investigated in duplicate by addition of 50 μl of each decomplemented hybridoma culture supernatant with 50 μl of a 0.5% RBC suspension of pooled, washed human OR_1R_2 or AB cells in saline. The mixtures were incubated for 90 min at room temperature prior to assessment of agglutination both visually and by microscopy. For indirect agglutination, the cells were washed and 10 μl rabbit antibody to mouse Ig (Dakopatts A/S, Denmark) added prior to a further 30-min incubation and examination for agglutination.

Enzyme-Linked Immunosorbent Studies

Indirect ELISA assays were performed with the hybridoma culture supernatants using Cooke M129B microtiter plates (Dynatech, Billingshurst, UK). In all cases, assays were performed wholly at room temperature and in duplicate.

Well homogenized intact cell membrane vesicle preparations (50 μl, 50-200 μg protein/ml) in isotonic phosphate-buffered saline (PBS), pH 7.4, with 1 mM $MgCl_2$ were incubated in microtiter wells pretreated with 50 μg/ml poly-L-lysine (Sigma) in PBS for 40 min. After 45-min incubation, the nonattached vesicles were removed and the attached vesicles then rapidly fixed with freshly prepared 0.1% glutaraldehyde for 3 min. Following washing with PBS, plates were incubated with 0.3% gelatin in PBS with 1 mM EDTA, pH 7.4, for one hour; subsequent washings and antibody dilutions were performed in this buffer. Microtiter plate wells were incubated with 50 μl of appropriate dilutions of hybridoma culture supernatants for one hour, washed three times, and then incubated for one hour with 100 μl peroxidase-conjugated rabbit antibody to mouse Ig (Miles) diluted 1:1000 followed by three more washes. Freshly prepared enzyme substrate, 0.4 mg/ml o-phenylenediamine (OPD; British Drug Houses, Poole, UK) in 0.1 M phosphate-citrate buffer, pH 5.5, with 0.013% hydrogen peroxide, was then added (100 μl) to each well. The colorimetric reaction was stopped by addition of 100 μl of 4 M sulfuric acid. Resultant color reactions were determined visually or by measurement of optical absorbance at 490 nm with a Dynatech MicroElisa reader.

Soluble protein antigens were used as targets in ELISA assays following attachment to microtiter plate wells by overnight incubation of 50-μl volumes in 0.05 M carbonate buffer, pH 9.6. Optimal coating concentrations (0.1-100 μg/ml) were determined by initial titration. The following human protein antigens were studied: pooled NHS, placental alkaline phosphatase (AP, type XXIV, 15 U/mg [Sigma]; and chromatographically purified grade, 150 U/mg [Miles]), serum albumin (HSA) and transferrin (Sigma), IgG (Kabi AB, Sweden), IgG fragments, IgM and IgA,[16] NaDOC-solubilized kidney plasma membrane and StMPM preparations.[3,14] For ELISA studies on reactivity of hybridoma culture supernatants with the above protein antigens, all washing and dilution procedures were carried out in PBS, pH 7.4, containing 0.05% polyoxyethylene sorbitan monolaurate (Tween 20, Sigma). Otherwise, ELISA was performed as described for plates coated with membrane vesicles except that, for studies on human Ig-coated plates, 0.5% NHS was included with the diluted peroxidase-conjugated antimouse Ig antibody in order to block any species cross-reactive specificities.

In all ELISA studies, polyclonal antiserum (anti-TA) taken from BALB/c mice immunized with human StMPM

preparations was used as a positive control at 1:200 dilution. Negative controls included wells with no attached antigen, and wells in which no hybridoma culture supernatant had been added or a comparable dilution of 10 μg/l mouse IgG (Miles) used instead.

Monoclonal Antibody Isotype

The isotype of various monoclonal antibodies was determined by double radial immunodiffusion[14] of hybridoma culture supernatants against goat antisera to mouse IgG or IgM (Cappel Laboratories, Cochranville, Pa) and goat antisera to mouse IgG subclasses (Meloy Laboratories, Springfield, Va).

RESULTS

Reactivity in Tissue Immunofluorescence

All 20 monoclonal antibodies reacted with term placental villous tissue in immunofluorescence to varying degrees of intensity (Table I). H315 and H317 demonstrated sequestration of reactivity solely to the trophoblastic component of placental villous tissue. H315 intensely stained the surface of villous syncytiotrophoblast as well as conglomerates within intervillous spaces presumed to be exfoliated microvilli aggregates and syncytial elements (Fig. 1a). On first trimester tissues, H315 sharply stained the microvillous membrane of villous syncytiotrophoblast (Fig. 1b). H317 stained term syncytiotrophoblast predominantly towards the apical surface in a more punctate distribution (Fig. 1c); this antibody stained a 27-week placenta but did not stain first trimester tissues (10–14 weeks).

H310 demonstrated pronounced cytoplasmic staining of both first trimester and term syncytiotrophoblast, but gave little staining of cytotrophoblast (Fig. 1d). Of the other antibodies, H301, H306, H307 (Fig. 1e), H308, and H316 were particularly reactive with the trophoblastic component of placental tissue compared with nontrophoblastic tissue. This staining, invariably including cytotrophoblast, was always cytoplasmic although often with a slightly greater staining intensity at the apical surface of syncytiotrophoblast. H304, H305, and H319 demonstrated comparable trophoblastic staining, but also with clear staining of elements within the mesenchymal stroma of placental chorionic villi. All antibodies that stained villous trophoblast also stained chorionic membrane epithelium (and, indeed, H316 demonstrated a more pronounced staining intensity on chorionic membrane than on villous trophoblast), whereas several of these antibodies (H301, H310, H315, H316, and H317) did not stain epithelial cells of amniotic membranes (Table I).

In studies of the reactivity of these antibodies on 24 separate human tissues other than placenta, several (H300, H303, H304, H305, H308, H309, H311, H312, H313, and H319) showed clear reactivity with multiple tissues and hence were designated as not trophoblast-specific (Table I). H301, H315, and H317 demonstrated no reaction with any other tissue and were provisionally designated as trophoblast-specific, although H301 was a weak specificity and gave slight nontrophoblastic staining in placental tissue. Of the other antibodies, H302, H306, H307, H310, H314, and H316 gave at best weak staining of no more than four nonplacental tissues. Of particular interest was H310: this was a strong specificity for trophoblast that also gave a weak diffuse staining of spleen and lymph node tissue, but did not stain amniotic membrane or any other human tissue.

Reactivity With Lymphocytes

Of the antibodies tested, H305, H306, H308, H310, H316, and H319 were reactive with human PBL; H308 and H319 were lymphocytotoxic (Table I). Results were consistent regardless of PBL donor, and no alloreactivity was noted. In all cases of reactivity on PBL manifested by immunofluorescence, the surface staining was speckled in distribution. H310 and H316 gave weaker PBL staining than the other positive monoclonal antibodies. H315 and H317 were clearly unreactive with PBL. No differences were noted for reactivity in immunofluorescence, or lack thereof, of these antibodies on PHA-stimulated PBL compared with resting PBL. No antibody that was tested (H301, H302, H305, H306, H307, H308, H310, H314, H315, H316, H317, H319) was inhibitory either to mitogenic stimulation of PBL by PHA or to allogeneic MLC reactions.

TABLE I. Reactivity of Monoclonal Antibodies With Cellular Antigens

Antibody	Tissue IF			Cell Suspension IF		Lymphocytotoxicity	RBC agglutination
	Placenta	Amnion	Other tissues[a]	PBL	MCF-7		
H300	+	NT	+	NT	NT	NT	NT
H301	wk	–	–	–	wk	–	–
H302	wk	NT	wk	NT	NT	–	–
H303	wk	NT	+	NT	NT	NT	NT
H304	+	+	+	NT	NT	NT	NT
H305	+	+	+	100%	NT	–	Indirect
H306	+	+	wk	>90%	+	–	Indirect
H307	+	+	wk	<10%	+	–	–
H308	+	wk	+	NT	+	60-80%	NT
H309	wk[b]	NT	+	NT	NT	NT	–
H310	+	–	wk	100%	+	0-50%, wk	–
H311	+	NT	+	NT	NT	NT	NT
H312	wk	NT	+	NT	NT	NT	NT
H313	+	NT	+	NT	NT	NT	NT
H314	wk[b]	–	wk	–	NT	–	–
H315	+	–	–	–	–	–	–
H316	wk	–	wk	100%	+	–	–
H317	+[b]	–	–	–	–	–	–
H318	wk[b]	NT	+	NT	NT	NT	–
H319	+	+	+	100%	+	100%	Direct

[a]Tissues studied included: umbilical cord, uterus, liver, kidney, spleen, two separate lymph nodes, brain, thymus, thyroid, intestine, skin, tongue, lung, stomach, pancreas, salivary gland, bronchus, myocardium, cervix, ovary, testis, breasts, and inflamed synovial membrane (rheumatoid). Positivity indicates reactivity with one or more of these tissues.

[b]Reactive with term tissue, but not first trimester tissue. All other antibodies stained both term and first trimester tissue.

wk: weak, NT: not tested, IF: immunofluorescence, PBL: peripheral blood leukocytes, RBC: red blood cells.

Reactivity With Erythrocytes

Of the antibodies tested, H305, H306, and H319 were reactive with human RBC (Table I). No difference was noted for reactivity on human AB or OR_1R_2 erythrocytes. Studies performed using a standard erythrocyte panel in the Liverpool Blood Transfusion Service showed that H305, H306, and H319 agglutinated virtually all RBC irrespective of the combination of RBC antigens carried on their surface.

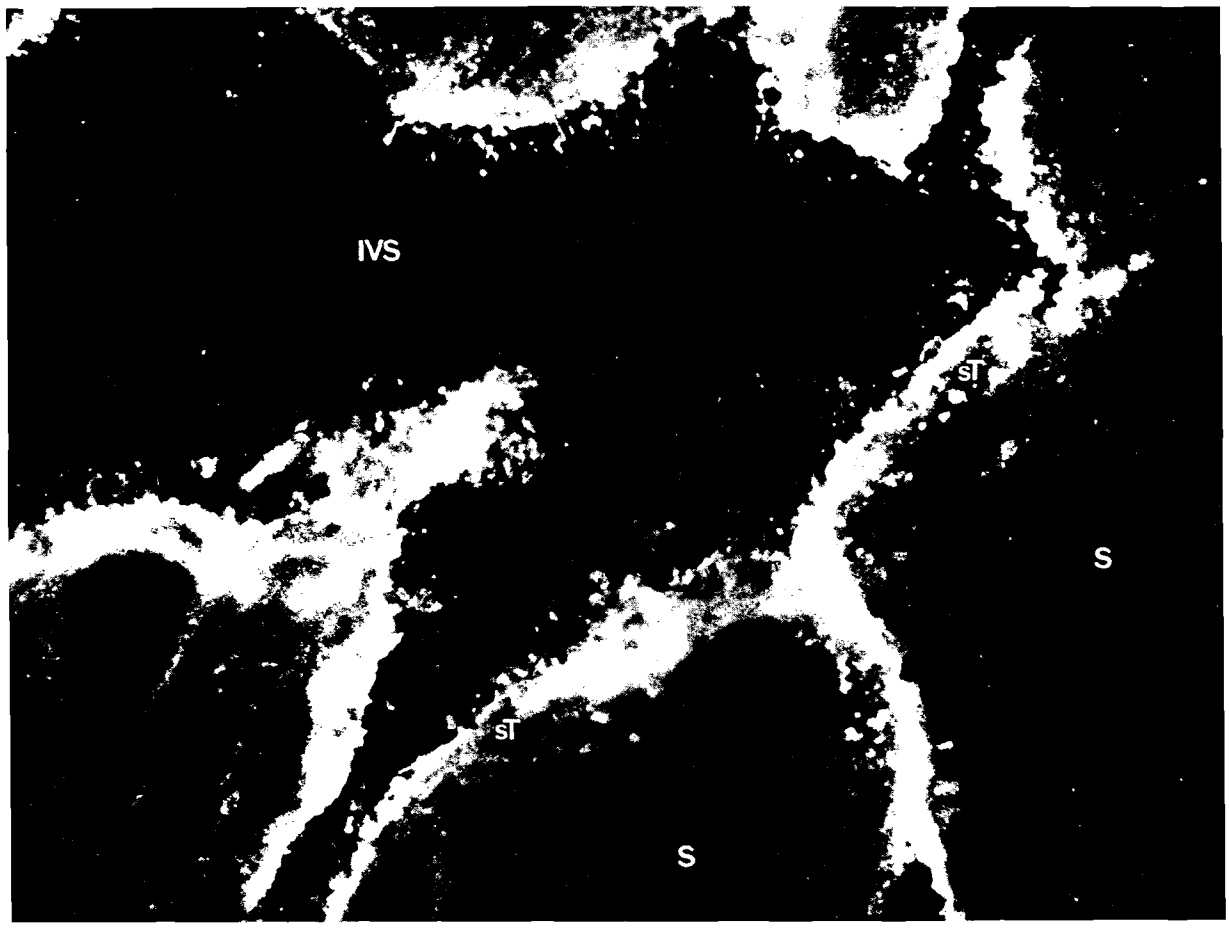
Reactivity With MCF-7 Breast Carcinoma Cells

Of the antibodies tested, all those that were reactive with PBL were also strongly reactive with MCF-7 breast carcinoma cells (Table I). In all cases, the surface staining was speckled in distribution (Fig. 2). As for PBL, H315 and H317 were also clearly unreactive with MCF-7 cells. However, H307 and, more weakly, H301 were reactive with MCF-7 cells but not with PBL.

Reactivity in ELISA

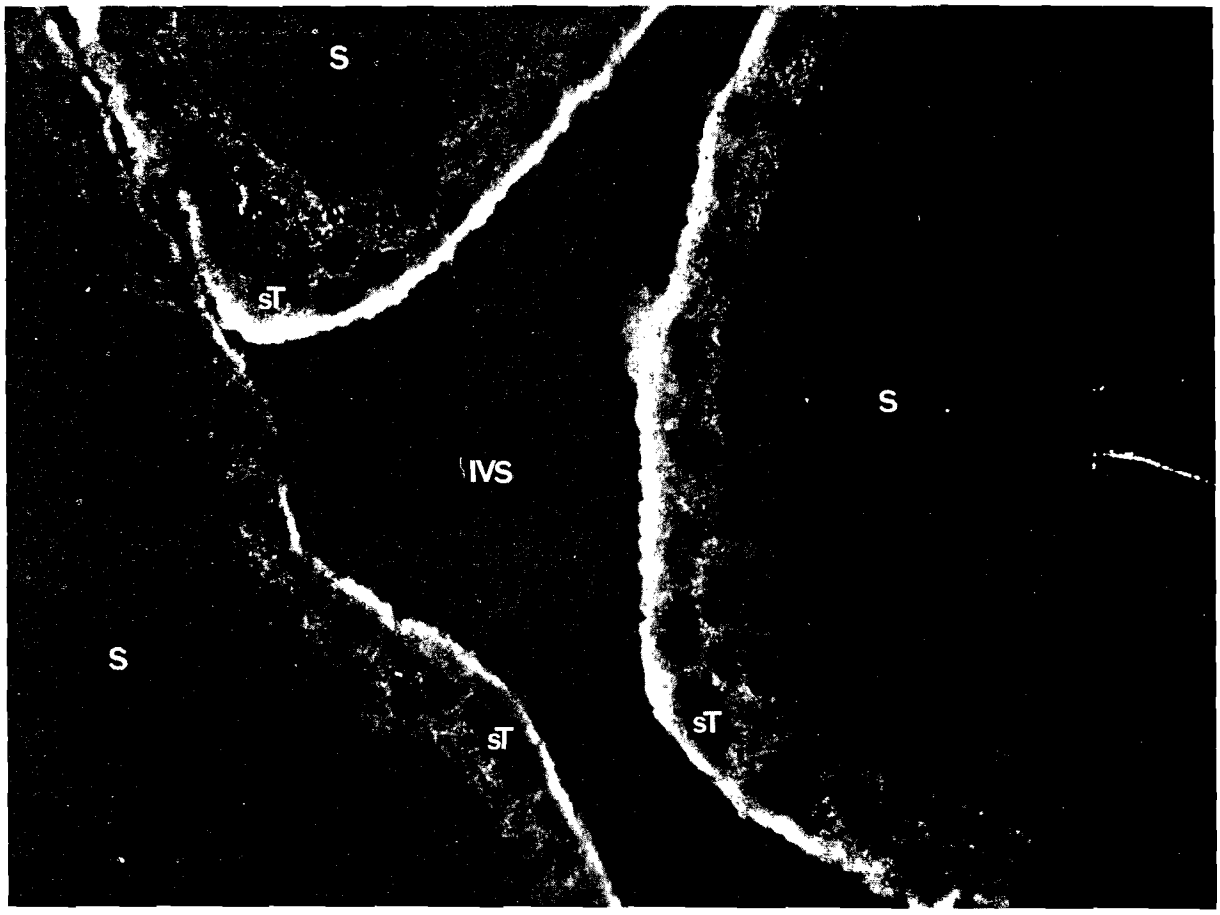
All monoclonal antibodies tested in ELISA were reactive to varying degrees with intact or NaDOC-solubilized term StMPM (Table II). Parallel studies using intact or NaDOC-solubilized kidney membranes confirmed the trophoblast-specificity of H315 and H317, whereas a lack of trophoblast-specificity of H301, H302, H305, H306, and H319 was also confirmed (Table II). These studies were compatible with H307 being predominantly trophoblast-reactive.

All 20 antibodies (H300–H319) were tested against NHS, AP, transferrin, HSA, and IgG. Four antibodies were positive against NHS; H309 and H318 were shown to be anti-IgG; H312 was shown to be a weak antialbumin specificity; and H303 was shown to be antibody to an NHS component other than those tested (Table III). No monoclonal antibody was reactive with AP or transferrin, although these specificities were demonstrated in the polyclonal anti-TA serum taken from immunized mice. Furthermore, no monoclonal antibody was consistently reactive with partially purified StMPM transferrin receptor preparations.[5] Both H318 and H309 were shown to react with human IgG and Fc fragments, but not with Fab, $F(ab')_2$, pFc′ fragments, or IgA and IgM. Hence, both these specificities would appear to be directed against antigenic determinants in the $C\gamma 2$ domain of IgG. This conclusion was confirmed by hemagglutination studies performed by Dr R. Jefferis (University of Birmingham) using sheep RBC sensitized with various human immunoglobu-



a

Fig. 1. Indirect immunofluorescence staining on cryostat sections of normal human placental villous tissue using monoclonal antibody followed by FITC-conjugated antimouse Ig: a) H315 on term placenta. Note staining of syncytiotrophoblast and of conglomerates within intervillous spaces, as well as lack of staining of nontrophoblastic stromal tissue. b) H315 on first trimester placenta. Note sequestration of reactivity to the microvillous surface of syncytiotrophoblast. c) H317 on term placenta. Note staining predominantly towards the microvillous surface of syncytiotrophoblast. d) H310 on first trimester placenta. Note cytoplasmic staining of syncytiotrophoblast and little staining of cytotrophoblast. e) H307 on first trimester placenta. Note cytoplasmic staining of both syncytiotrophoblast and cytotrophoblast. Code: IVS, intervillous spaces; S, villous stroma; sT, syncytiotrophoblast; cT, cytotrophoblast.



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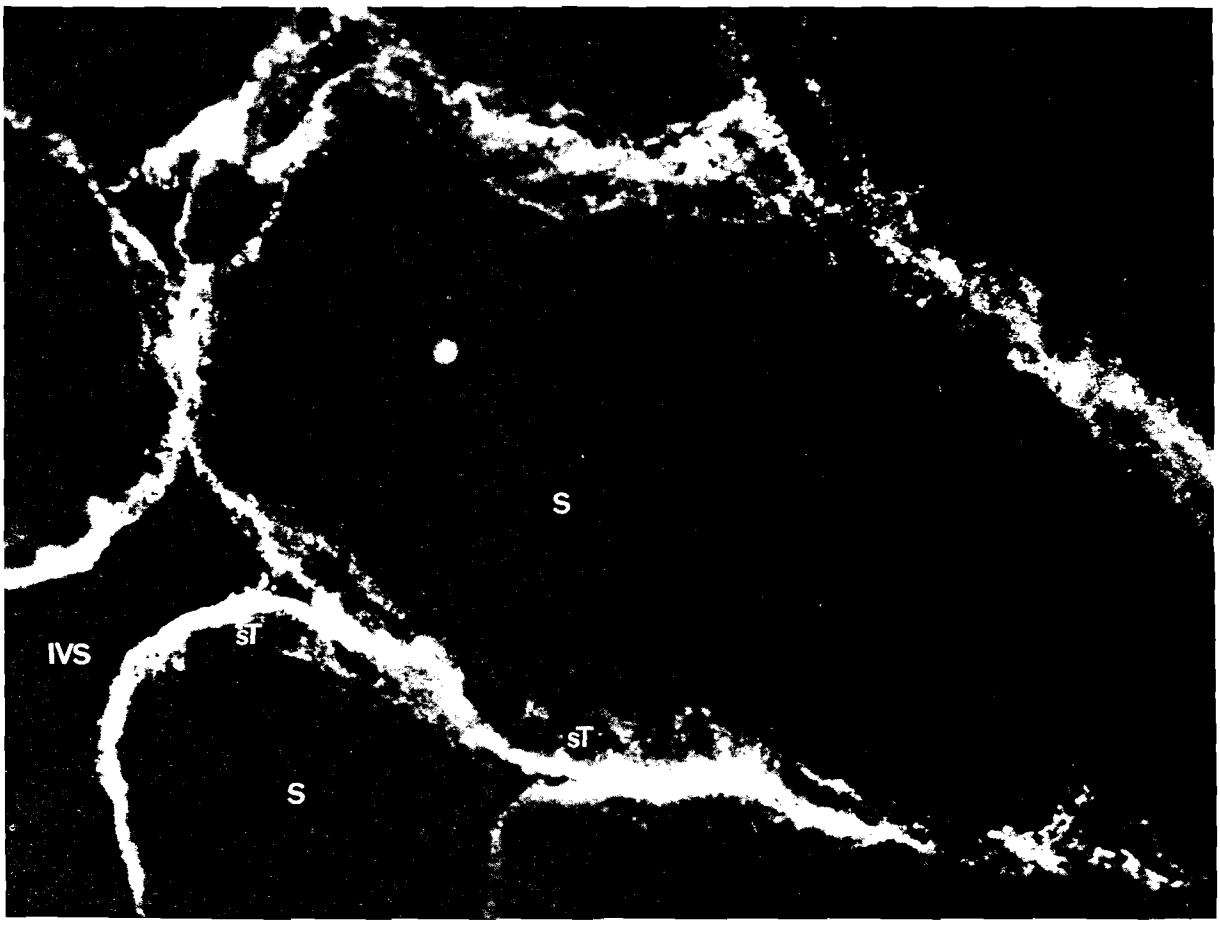

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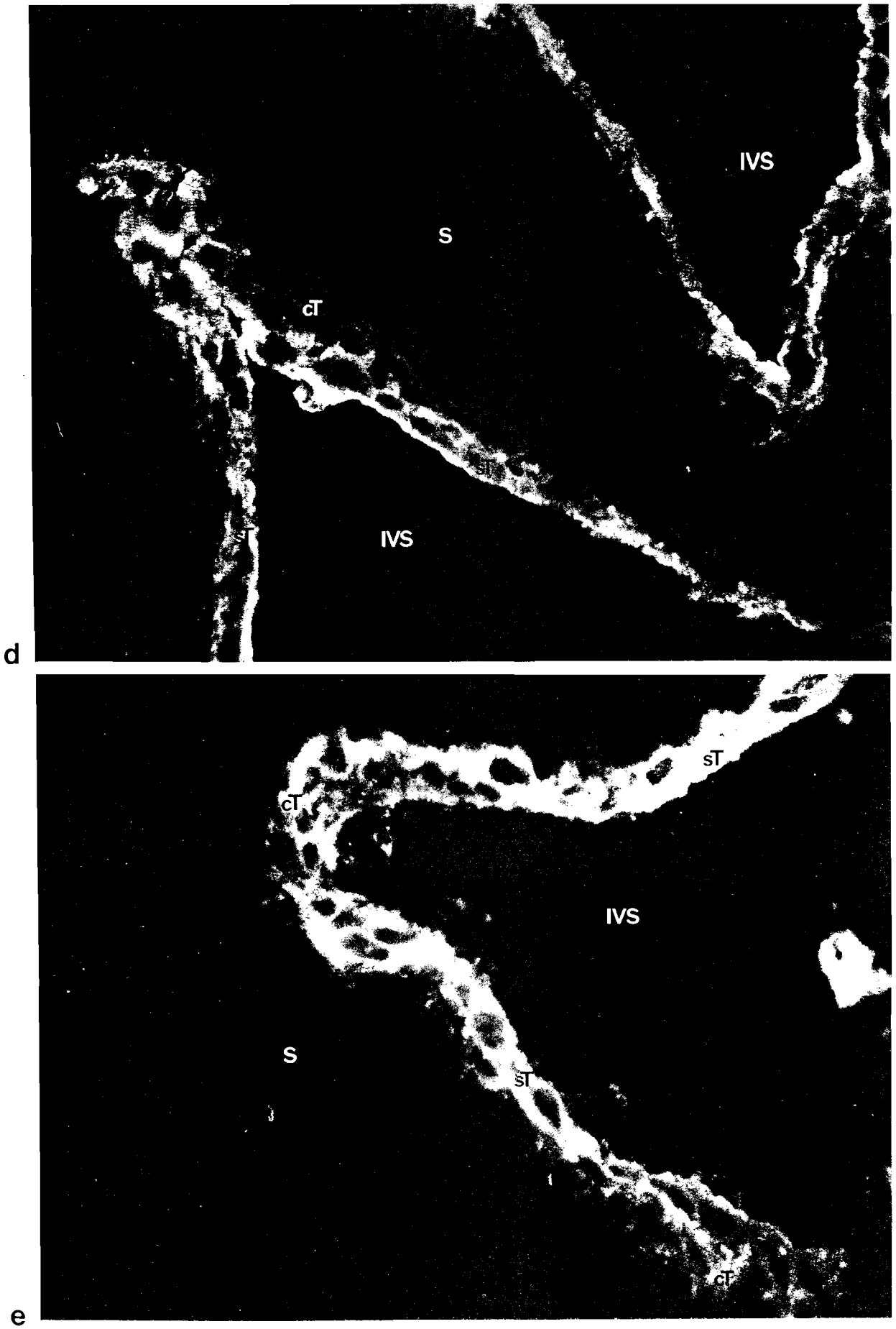
Fig. 1. (continued)



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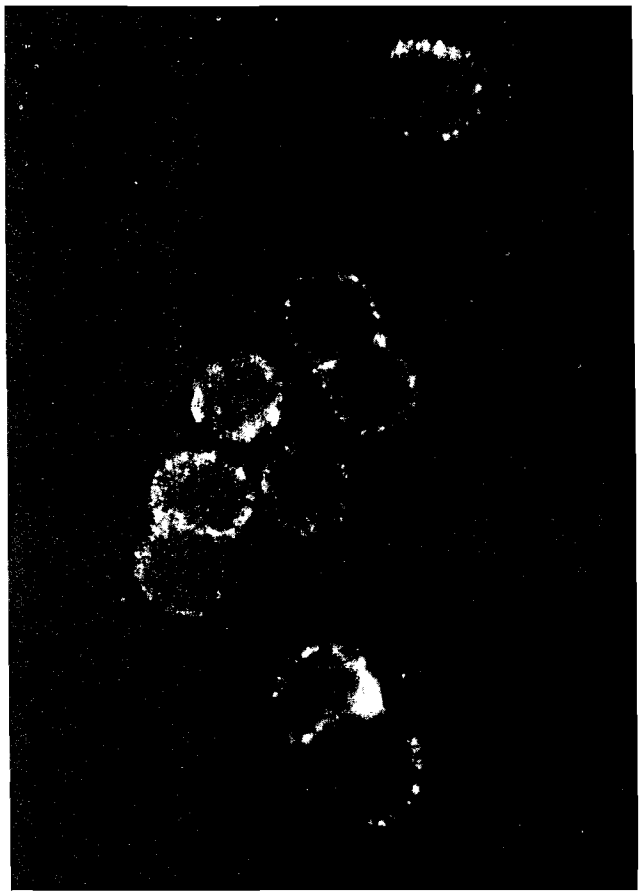

Fig. 2. Indirect immunofluorescence staining on the surface of MCF-7 human mammary carcinoma cells using H310 monoclonal antibody followed by FITC-conjugated antimouse Ig. Note speckled distribution.

TABLE II. ELISA Analysis of Reactivity of Monoclonal Antibodies With Intact Membranes or NaDOC-Solubilized Membrane

	Antigen			
Antibody	Intact StMPM	NaDOC-sol StMPM	Kidney PM	NaDOC-sol Kidney PM
H301	wk	+	wk	–
H302	wk	wk	wk	–
H305	+ +	+ +	+ +	+
H306	+	+	wk	–
H307	+	wk	–	–
H308	wk	+	–	–
H309	wk	+	wk	–
H310	+	+	–	–
H315	+	+ +	–	–
H316	+	wk	–	–
H317	+	+	–	–
H318	wk	wk	wk	–
H319	+ +	+ +	+ +	+
Anti-TA	+ +	+ +	+	+

PM: plasma membrane, StMPM: syncytiotrophoblast microvillous plasma membrane, NaDOC-sol: sodium deoxycholate-solubilized, wk: weak.

TABLE III. ELISA Analysis of Reactivity of Monoclonal Antibodies With Soluble Protein Antigens

	Antigen				
Antibody	NHS	AP	Trf	HSA	IgG
H303	+	–	–	–	–
H309	+	–	–	–	+
H312	wk	–	–	wk	–
H318	+	–	–	–	+
Anti-TA	+	+	+	+	+

wk: weak, NHS: normal human serum, AP: alkaline phosphatase, Trf: transferrin, HSA: human serum albumin

lins and fragments.[17] This group also showed that H309 reacted with the human IgG_1, IgG_2, and IgG_3 subclasses, whereas H318 reacted with IgG_1, IgG_2, IgG_3, and IgG_4 subclass proteins. Finally, in ELISA, H318 and H309 were demonstrated not to be directed against the carbohydrate portion of IgG since their reactivity was not abolished by pretreatment of IgG with periodic acid (0.5 mg/ml IgG incubated with 0.35 mM periodic acid for 30 min at room temperature).

Monoclonal Antibody Isotype

The Ig classes of various of the monoclonal antibodies were determined, together with IgG subclassing when unambiguous results were obtained, and are shown in Table IV.

DISCUSSION

The specificities of 20 separate monoclonal antibodies produced against human term StMPM preparations have been investigated. The majority of these antibodies were demonstrated to be not trophoblast-specific. However, two hybridomas (H315 and H317) were shown to secrete monoclonal antibodies that were strongly reactive with syncytiotrophoblast but gave no reaction with PBL, RBC, or 22 separate adult human tissues. Furthermore, these antibodies did not react with amniotic membrane or umbilical cord. A very recent study by Sunderland et al[18] has also demonstrated the presence of trophoblast-specific antigens using monoclonal antibodies. Hence, this use of monoclonal antibodies has confirmed previous studies favoring the identification of trophoblast-specific antigens using polyclonal heterologous antisera.[14,19,20] These monoclonal antibodies, however, have removed the necessity for the tedious adsorption procedures that had been required for polyclonal antisera to render them specific.[14,15,20] Furthermore, the introduction of monoclonal antibodies will facilitate the development of sensitive immunoassays for trophoblast fragments that have broken away into the maternal circulation; such assays may have some clinical usefulness. Since the monoclonal antibodies have been derived following initial heterologous immunization, there is no a priori reasoning that their corresponding antigens may have any direct immunological function in homologous pregnancy. However, these monoclonal antibodies will greatly assist improved purification for individual trophoblast surface components, and hence also assist their detailed biochemical and functional investigation.

The H315 antigen has been shown to be present on first trimester trophoblast, unlike the H317 antigen; thus, these two antigenic determinants reside on separate molecular structures. It would clearly be of interest to determine the occurrence of the H315 antigen on cellular elements involved earlier in embryogenesis (eg, sperm, blastocyst), especially since this antigen could be relevant to experimental concepts for the immunoregulation of fertility. The occurrence of the H315 and H317 antigens in pregnancy serum is also unknown, although neither of these monoclonal antibodies (nor any of the other monoclonal an-

TABLE IV. Isotypes of Monoclonal Antibodies

IgG (subclass not determined)	:	H301, H302, H305, H307, H308, H309, H312, H316, H317, H318
IgG1	:	H306, H310
IgG2b	:	H315
IgM	:	H319

The remaining monoclonal antibodies were not tested.

tibodies) reacted preferentially in ELISA with plate wells coated with term pregnancy sera rather than NHS. This would indicate that the relevant antigen is not found in substantial quantities in term pregnancy sera (as, for example, SP1).

One monoclonal antibody (H310) appears to define an antigenic determinant shared only by trophoblast and lymphocytes. Other antibodies were reactive with lymphocytes, but these were also reactive with other nontrophoblastic cell types. The concept of shared lymphocyte-trophoblast antigens has been proposed previously,[20-22] and it has been thought that these may play some role in the control of immunological interplay between mother and fetus. The description using a monoclonal antibody of an antigenic determinant restricted to trophoblast and lymphocytes would appear to strengthen this concept, although no antibody that was tested had any inhibitory effect on mitogenic or allogeneic stimulation of lymphocytes. This is unlike the situation for polyclonal rabbit antisera to human trophoblast antigens which have been reported to inhibit allogeneic MLC responses.[21] No selective reactivity was noted for any monoclonal antibody with different donor PBL, indicating that none of these specificities was directed against allotypic epitopes of cell surface structures.

All monoclonal antibodies reactive with PBL were also reactive with the MCF-7 human mammary carcinoma cell line. Furthermore, two specificities (H301 and H307) were reactive with MCF-7 cells but not with PBL. Of these, H301 was a weak specificity and its apparent selection for MCF-7 cells may represent minor variation in epitope density of the relevant antigenic determinant on different cells. Although H307 also reacts with amniotic epithelium and gives weak diffuse staining of several other normal tissues, its clear reactivity with MCF-7 cells would indicate that it may merit further study in reaction on different human cultured cell lines. Indeed, previous studies have indicated that heterologous polyclonal antisera to human trophoblast antigens may recognize a cell surface antigenic determinant also expressed on various carcinoma and transformed cell lines[4,23,24] as well as on carcinoma cells in sections of malignant tissue.[15,25] This has been particularly demonstrated for breast carcinoma cells. However, preliminary studies have failed to show any of the monoclonal antibody specificities (including H301 and H307) to be useful in selective immunohistological identification of malignant cells in bronchial and breast carcinoma tissue sections.

Blood group antigens are thought to be absent from StMPM[1,2] and therefore it was surprising that three antibodies (H305, H306, and H319) reacted with human RBC. However, all of these antibodies reacted with PBL and, on detailed study, showed pan-reactivity with a wide panel of different human RBC types. It would therefore appear most likely that some or all of these antibodies may be directed against common carbohydrate groupings within stem structures of glycosylated cell surface components.

It was also of interest to attempt to determine the specificity of the other monoclonal antibodies raised against StMPM in order to gain some insight into the relative distribution of StMPM-associated components recognized by heterologous immunization. Interestingly, no antibody was reactive with placental alkaline phosphatase, although this is a strong immunogen in polyclonal immunization of rabbits with StMPM preparations.[14] Two antibodies (H309 and H318) were reactive with the $C\gamma 2$ domain of IgG and one (H312) was reactive with albumin. This is in accord with previous data showing a small but significant amount of IgG and albumin to be consistently associated with isolated StMPM preparations.[3] One other antibody (H303) reacted with an NHS component that was not transferrin. Preliminary data have also indicated that none of these antibodies is reactive with either placental ferritin[26] or the $Fc\gamma$ receptor structure[27] associated with isolated StMPM. Furthermore, none was reactive with a partly purified transferrin receptor preparation[5] in which the major nonreceptor protein was its complementary ligand, transferrin. The absence of any reactivity with the transferrin receptor is compatible with the absence of selective reactivity with rapidly proliferating cells (MCF-7 and PHA-stimulated PBL). Nevertheless, it is known that the transferrin receptor is an important component of StMPM preparations[6] and is immunogenic on heterologous immunization to produce polyclonal antisera[28]; monoclonal antibodies have also been produced to this structure on other cell types.[29,30]

ACKNOWLEDGMENTS

We would like to thank Dr R. Jefferis (University of Birmingham) for investigating the anti-Ig specificities of H309 and H318, and Mr M. Chapman (Liverpool Blood Transfusion Service) for detailed serological analysis on three of these monoclonal antibodies. We are also extremely grateful to Peter Brown for helpful criticisms, to Ms P. Evans for testing several of these monoclonal antibodies in lymphocytotoxicity and lymphocyte responsiveness assays, and to Dr C. A. St. Hill for assistance in collection of substrate tissues.

NOTE ADDED IN PROOF

None of these monoclonal antibodies has been found to be reactive in ELISA with purified HCG (Sigma), SP1 (a gift from Dr P. D. G. Dean, Department of Biochemistry, Liverpool University), PAPP-A, or α_2-macroglobulin (gifts from Dr R. G. Sutcliffe, Institute of Genetics, Glasgow University).

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